

**Functional analysis of PP17b and PP20 soluble  
placental proteins**

**A PP17b és PP20 szolubilis placentáris fehérjék  
funkcionális vizsgálatai**

**Szabolcs Bellyei , MD**

**PhD thesis**

**PhD tézisek**

**Medical University of Pécs  
Department of Biochemistry  
and Medical Chemistry**

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## Introduction

As their name suggests, pregnancy-related proteins were discovered through comparative examinations of pregnant and nonpregnant samples. Of the placental hormones and enzymes, human chorionic gonadotropin (hCG), human placental lactogen (hPL) and heat-stable alkaline phosphatase have been known for several decades. Since the '70's the number of these pregnancy-related proteins has grown steadily. In addition to three fetal and seven pregnancy proteins, Hans Bohn has isolated 20 solubilized or membrane associated placental proteins (MPs) and 26 soluble placental tissue proteins (PPs).

Women's Clinic and jointly Institute of Biochemistry and Medical Chemistry of the University of Pécs has been involved in the collaboration research of the pregnancy-related oncofetal proteins for thirty years. During the years of collaboration the team has performed the isolation, and the basic and applied research study in normal and pathologic circumstances of 9 novel proteins, and developed new measuring procedures. Since 1997 the team have been dealing with the sequential, structural, functional and expressional analyses of 8 placental proteins (PP13, PP17a, PP17b, PP18a, PP18b, PP20, PP23 and PP25) with genetical and proteomical methods.

The research group discovered the PP17 protein family at the millennium. Two members of the protein family could be cloned and sequenced (PP17a: GenBank Accession No. AF051314, AF051315; PP17b: GenBank Accession No. AF055574; PP17c and PP17d). The team have started the functional analysis of the PP17 protein family. The PP20 protein was not identified before, we knew only slightly more than a decade. PP20 is composed of two identical 27 kDa subunits. The carbohydrate content of PP20 was found to be relatively low (3.0% by weight) and the average amount of PP20 present in one human term placenta was found to be 0.5 mg.

## **Study objectives**

1. Databank search to reveal the expression, structure and regulation of the PP17 gene. Multiple sequence alignment, to prove to be a member of the growing lipid storage droplet protein family.
2. Immunofluorescence microscopy and protein sequencing to present the evidence for the association of PP17b to lipid droplets and milk lipid globule membranes.
3. Using a HeLa cell model to show the importance of protein kinase A and protein kinase C dependent pathways for the regulation of PP17 gene expression, and the effect of the phase of cell cycle, differentiation and apoptosis on expression of this gene.
4. Isolation of the cDNA of PP20 and analysis of the nucleotide and deduced amino acid sequences.
5. Databank search to demonstrate the genomic localization, structure and regulation of PP20 gene
6. Identification of PP20 by mass spectrometry
7. Analysis of PP20 / hTPK1 enzymatic activity by using HPLC-MS with electrospray (ESI) ionisation
8. Expression and localization of PP20
9. Construction of the comparative 3D model of PP20

## General Conclusions

1. GenBank analysis of EST clones underlines that alternatively spliced PP17a occurs mainly in steroidogenic tissues, while PP17b is synthesized in almost all types of tissue, especially in placenta and epithelial origin tumors.
2. Sequence data show high level sequence similarity at their N-termini between PP17b and neutral lipid droplet associated proteins including perilipins and adipophilin, which latter was also involved in adipose cell differentiation. Taken altogether, a comparison of PP17b and its gene to perilipins and adipophilin, members of the “PAT domain gene family,” similar exon structures, sequence homology and many common transcription factor regulatory sequences in the promoter regions were found, suggesting their common genetic origin and functional similarities.
3. With different techniques based on immunological reactions, considerable evidence was obtained to the effect that PP17b/TIP47 was a neutral lipid droplet associated protein, which also occurs in significant quantities in milk lipid globule membranes. Because of the controversy in the literature on its function, to avoid possible immunological cross-reactivity, a very specific independent technique, MALDI-TOF MS analysis was used, and both PP17 variants – PP17b most markedly – were proved to bind to the surface of neutral lipid droplets. Furthermore, our previous data showed that both PP17a and PP17b could aggregate even in the presence of low concentrations of SDS, raising the possibility that these proteins could be involved in the formation of different-size

lipid droplets. By binding to lipid micelles and having self-aggregating properties, PP17 variants could facilitate lipid droplet aggregation, which is clearly detectable in the case of milk lipid globule membranes. This property of PP17b indicates its function as a neutral lipid droplet associated protein and its involvement in lipid droplet formation/mobilization, in accordance with its possible function in cell and tissue differentiation.

4. With computer analysis of its 5' up-stream sequence, several transcription factor binding sites were identified, including mostly proliferation and / or apoptosis regulators, embryo- and organogenic factors, proto-oncogenes or their targets, which also points to the possible complex PP17 gene regulation.
5. Induction of apoptosis and differentiation indeed up-regulated PP17 expression, while kinase cascade inhibition led to a transcription factor activation block on the induction of PP17 expression, providing evidence for the importance of those transcription factors in PP17 gene regulation. These data also indicate that PP17b could play an important role in tumor cell development and differentiation. Since providing rich lipid supply to cells induced lipid droplet formation and PP17b overexpression, this indicates that PPAR $\gamma$  could have a role in the regulation of PP17 expression. Furthermore, these data suggest that the main function of PP17a and PP17b is involvement in lipid droplet formation and in rearrangement of lipid membranes, which processes could also be important in cell differentiation and division. The high concentration of PP17b in milk lipid globule

membranes indicates its potential role in exporting lipid droplets and membranes.

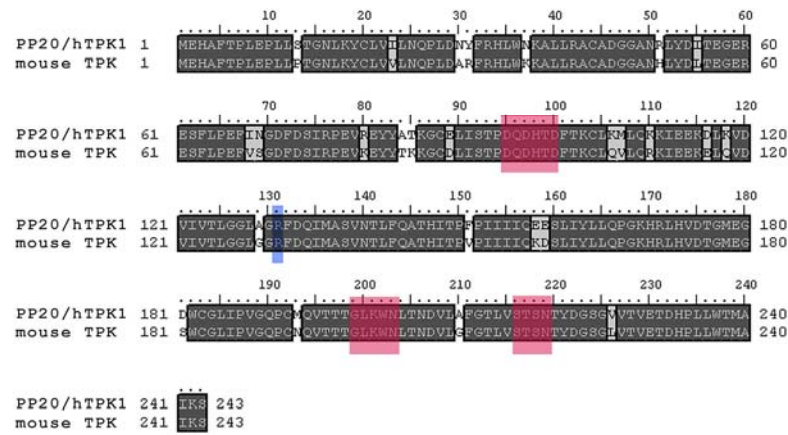
6. In the case of several previously known “placental proteins,” which turned out to have a general function in different human tissues, more specific structural or functional names were given, such as galectin-13 (PP13), glycodelin (PP14) or branched-chain aminotransferase (PP18). As (1) PP17b is synthesized ubiquitously, while PP17a is found mainly in steroidogenic tissues; (2) both PP17 variants are generally involved in lipid droplet formation, like alternatively spliced perilipins, which were shown to bind either to steroid or neutral lipid droplets; (3) neither the name “*placental protein 17b (PP17b)*” nor “*tail-interacting protein of 47 kDa (TIP47)*” gives the appropriate information on the structure, function, regulation, or the origin of this protein; (4) there is still a lack of an official name for the “PP17/TIP47” gene; and (5) there is a common need to elucidate this controversial situation, it is therefore now proposed that the PP17 variants be renamed to *sandrin A (PP17a)* and *sandrin B (PP17b)* (Steroid And Neutral lipid DRoplet-associated proteIN), and their gene to SNDR.
7. Four cDNAs have been isolated from placental library encoding the 243 residue-long protein, having two variants (27 kDa and 54 kDa). The 54 kDa variant was verified to be a dimer. By its primary nucleotide sequence, PP20 proved to be identical to human thiamin pyrophosphokinase (hTPK), as confirmed by protein sequence analysis.

8. GenBank search information revealed PP20 / hTPK gene was located on chromosome 7q34-q36 and was predominantly expressed in the placenta. The genomic sequence contained 9 exons, not 8 as published previously when the exon division and 5'-upstream region of the gene were not yet resolved
9. Analysis of the 1kb promoter region showed numerous putative transcription factor binding sites, which might be responsible for the ubiquitous PP20 / hTPK expression. This may also be in accordance with the presence of the protein in tissues responsible for the regulation of the exquisite balance between cell division, differentiation and survival.
10. TPK activity of the purified and recombinant protein was proved by mass spectrometry.
11. PP20 / hTPK was found in all human normal and tumorous adult and fetal tissues in nearly equal amounts, but not in sera.
12. By immunohistochemical and immunofluorescent confocal imaging methods, diffuse labelling in the cytoplasm of the syncytiotrophoblasts and weak staining of the trophoblasts were observed, and the amount of PP20 / hTPK decreased from the first trimester to the end of gestation.
13. A 3D model of PP20 / hTPK was computed (PDB No: 1OLY) by homology modelling. A high degree of structural homology showed that thiamin binding-site was highly similar to that of mouse enzyme, but highly different from the bacterial ones.

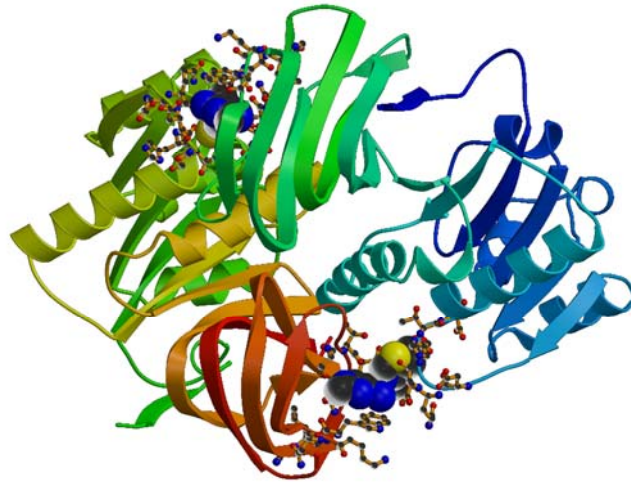


Comparison of the catalytic centre sequences revealed differences, raising the possibility of designing new drugs which specifically inhibit bacterial and fungal enzymes without affecting PP20 / hTPK and offering the possibility for safe antimicrobial therapy during pregnancy.

**Fig. A.**



**Fig. B.**



**Fig. 3D homology modelling and sequence alignment of PP20 / hTPK to mouse TPK. (A)** Identical residues in PP20 / hTPK and mouse TPK were shaded dark grey, conservative changes in light grey, the thiamin binding site in red and the suspected ATP binding site in blue. **(B)** 3D model of the PP20 / hTPK homodimer enzyme was constructed by homology modelling with MOLSCRIPT [20] based on the mTPK crystal structure. The 3D model was rendered by Raster3D. The peptide backbones were represented as ribbons, the thiamin binding-sites as balls and sticks, and thiamins as spacefills.

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#### Other Presentation

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